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13. (Amended) The method according to claim 1 wherein the culture medium further comprises at least one agent [factor] selected from the group consisting of TNF- α , G-CSF, IL-1 [and] or IL-3 [is present in the culture medium].

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22. (Amended) The d[D]endritic cell precursors produced by [prepared according to] the method of claim 1.

REMARKS

Claims 1-23 are pending in this application. Claims 14-21 have been withdrawn from consideration. Claims 1-13 and 22-23 stand rejected. Reconsideration of the claims as amended is respectfully requested.

Rejection of Claims Under 35 U.S.C §112 . Second Paragraph

Claims 1-13 and 22-23 stand rejected under 35 U.S.C. §112, second paragraph because the Examiner contends that they are indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as invention. In particular, the Examiner contends that the claim preamble indicates the claim is drawn to a method of producing proliferating cells, when mature cells actually result from the claimed method.

As suggested by the Examiner, Applicants have amended the preamble of claim 1 to recite "A method of producing a population of mature dendritic cells from proliferating precursors". Step (b) of claim 1 has also been amended to conform to the

Examiner's suggestion. For clarity, claim 13 has been amended to recite that the culture medium must further comprise an agent selected from the group recited.

Claims 2, 4 and 5 have been amended to recite "said factor" instead of "said agent", thereby obviating the rejection regarding lack of antecedent basis. The minor errors and informalities in claims 7 and 22 have also been corrected. As amended, the claims are believed to conform to the statute. Reconsideration is respectfully requested.

Rejection of Claims 1-5 and 10 Under 35 U.S.C. 102(b)

Claims 1-5 and 10 stand rejected under 35 U.S.C. 102(b) as being anticipated by Markowicz et al. Specifically, the Examiner contends that

"Markowicz et al. teach a method of producing a population of dendritic cells. The method comprises providing a population of cells from human peripheral blood which comprises dendritic cells. The dendritic cells were then cultured in microwells containing supplemented RPMI-1640 medium, 10% heat inactivated human serum and 100 U/ml of GM-CSF. Markowicz et al. further teaches that the culture medium could additionally be supplemented with IL-4."

Applicants traverse this ground of rejection.

For a finding of anticipation each element of the claimed invention must be present in a single reference. Markowicz et al. relates to the effects of GM-CSF on the morphology and viability of dendritic cells isolated from human peripheral blood. Markowicz et al. reports that GM-CSF promotes dendritic cell survival and induces dendritic cell differentiation. However, Markowicz et al. does not teach or suggest that these cells proliferate in culture or disclose conditions for allowing dendritic cells to proliferate in

culture. In fact, Markowicz specifically teaches away from applicants invention by teaching that GM-CSF does not cause proliferation of dendritic cells:

"At any given concentration of the cytokine, however, the total number of viable cells as well as the number of branched cells per well remained stable over time, suggesting that GM-CSF does not cause DC to divide and proliferate."

(Markowicz pages 958-959.)

As Markowicz et al. does not disclose methods for culturing proliferating dendritic cell precursors, the reference fails to anticipate the claimed invention.

Rejections of Claim 22 Under 35 U.S.C. §102 or Under 35 U.S.C. §103

The Examiner also asserts, that the Markowicz et al. reference renders Claim 22 *prima facie* obvious. Applicants traverse this ground of rejection.

As stated above Markowicz et al., relates to the affects of GM-CSF on the morphology and viability of mature dendritic cells. However, as stated above, Markowicz et al. does not teach that their dendritic cells proliferate in culture or that additional agents should be added that inhibit the maturation or proliferation of non-dendritic cell precursors. Therefore, Markowicz et al. does not teach or suggest the instantly claimed method which is directed towards methods of culturing and proliferating dendritic cells *in vitro* in the presence of factors that inhibit maturation of non-dendritic precursor cells to produce sufficient quantities of mature dendritic cells to be useful as adjuvants or for producing antigens. Therefore, withdrawal of the rejection and reconsideration are respectfully requested.

Rejection of Claims 6 and 11-12 Under 35 U.S.C. §103

Claims 6 and 11-12 stand rejected under 35 U.S.C. §103 because the Examiner contends they are unpatentable over Markowicz et al. as applied to claims 1-5 and 10 above and further in view of Jakoby et al. Specifically, the Examiner contends:

"Markowicz et al. is relied upon for the reasons discussed *supra* teaches utilization of 100 U/ml of GM-CSF and IL4. Markowicz et al. differs from the claimed invention by not specifically indicating the exact concentration of IL4 utilized and also by the utilization of slightly less concentration levels of GM-CSF from that which is specifically claimed. However, it is well known in the art to adjust the concentration level of culture medium additives in order to optimize the experimental conditions for the particular cell type being cultured. Jakoby et al., on pages 75-77, teach that it is well known in the art of cell culture to "tailor media" in order to optimize the experimental conditions. Each culture system requires examination of the particular conditions that are best for the type of cell being studied by the investigator. Further, each component of the system identified as a result-effective variables, has its well recognized advantages for the purposes of optimizing the experimental conditions."

Applicants traverse this rejection for the reason stated below.

Jakoby et al. relates to general methods of culturing cells. Jakoby discusses types of culture systems, requirements for growth and some generalized standards for tissue culture. Jakoby et al. does not discuss methods of culturing proliferating dendritic cells or the requirements, such requirements are found only in applicants' disclosure. In fact, Jakoby et al. recognizes that the general cell culture methods provided cannot necessarily be extrapolated to all cell types (see page 93, under Future Prospects). Therefore, Jakoby does not provide a disclosure which remedies the differences of Markowicz et al. as discussed

above. Hence, the invention is not rendered obvious by this reference either alone or in combination with Markowicz et al. Reconsideration and withdrawal of this rejection is respectfully requested.

Rejection of Claim 7 and 13 Under 35 U.S.C. §103

Claim 7 and 13 are rejected under 35 U.S.C. §103 as being unpatentable over Markowicz et al. as applied to claims 1-5 and 10 above and further in view of Koch et al. Specifically, the Examiner contends that:

"Koch et al. teaches that new insights into the biology of dendritic cells (DC) came from studies of murine-epidermal Langerhans cells *in vitro*. Koch et al. indicates that such studies indicate LC in the skin and DC in other non-lymphoid tissues represent precursors or immature elements of the dendritic cell system. Koch et al. teaches that the addition of TNF-alpha to murine epidermal Langerhans cells in culture allows such cells to maintain their viability. Therefore in view of the teachings of Koch et al. one of ordinary skill in the art would have a reasonable expectation of success in maintaining viability of dendritic cells when TNF-alpha is added to the dendritic cell culture. Accordingly, one of skill in the art would have a reasonable expectation of success in adding TNF-alpha to the dendritic cell culture of Markowicz et al."

Applicants traverse this rejection for the reasons discussed below.

The Examiner cited Koch et al. as disclosing the use of TNF- α in culturing dendritic cells. Koch et al. relates to the use of TNF- α in cultures of murine epidermal Langerhans Cells. Koch et al. does not relate to the production of mature dendritic cells from proliferating cultures of dendritic cells. Furthermore, Koch et al. neither teaches nor suggests the culturing of mature dendritic cells from proliferating dendritic cell precursors or the use of factors in such a culture which inhibit non-dendritic cell precursors. Therefore,

Koch et al. does not remedy the deficiency of Markowicz et al. as discussed above.

Withdrawal of this rejection is respectfully requested.

Rejection of Claims 8-9 and 23 Under 35 U.S.C. §103

Claims 8-9 and 23 stand rejected under 35 U.S.C. §103 as being unpatentable over Markowicz et al. as applied to claims 1-5 and 10 above and further in view of Voorhis et al. or Ruley et al. Specifically, the Examiner contends that:

"Voorhis et al. teach that human dendritic cells may be cultured in 5-10% fetal calf serum. Furthermore, it is well known in the animal culture field to utilize cord blood serum in animal cell cultures (See Ruley et al. U.S. Patent 5,364,783, column 22, lines 21-27). Therefore it is merely a matter of judicious selection on the part of the skilled artisan to utilize fetal calf or cord blood serum as opposed to human serum. Additionally, it is well known in the art to utilize anywhere from 1-20% of serum in animal cell cultures. Utilization of a particular concentration within that range is deemed merely a matter of routine optimization which is well within the purview of the skill artisan."

Applicants traverse this ground of rejection for the reasons discussed below.

Van Voorhis et al. relates to the enrichment and characterization of human dendritic cells from peripheral blood. Van Voorhis et al. does not teach or suggest proliferation of human dendritic cell precursors *in vitro*, nor does it teach nor suggest that additional factors that inhibit the maturation or proliferation of non-dendritic cell precursors should be added to cultured medium. Ruley et al. relates to retrovirus promoter-trap vectors and does not even discuss or teach culturing of dendritic cells or any conditions under which proliferating dendritic cells per se should be cultured. Absent such disclosures, neither Van Voorhis or Ruley can render the claimed invention obvious.

As discussed above, Markowicz et al. also does not teach obtaining mature dendritic cells from proliferating dendritic cell precursors or teach that additional factors such IL-4 or IL-13 should be added to the culture to inhibit the maturation or proliferation of non-dendritic cell precursors. Absent such disclosures, none of the references can render the claimed invention obvious either alone or in combination. Reconsideration and withdrawal of the 35 U.S.C. § 103 rejection is respectfully requested.

Rejection of Claims 1-6, 8-9, 13 and 22 Under 35 U.S.C. §103

Claims 1-6, 8-9, 13 and 22 are rejected under 35 U.S.C. §103 as being unpatentable over Hueffler et al. taken with Sallusto et al.

Hueffler et al. reports the use of GM-CSF and IL-1 in mediating the maturation of murine epidermal Langerhans cells into immunostimulatory dendritic cells. While Hueffler et al. reports the use of GM-CSF and an interleukin in the maturation of dendritic cells *in vitro*, it neither teaches nor suggests that GM-CSF can enhance the proliferation of precursor dendritic cells *in vitro* or that interleukin can inhibit the maturation or proliferation of non-dendritic cell precursors. In fact Hueffler et al. specifically states on page 701 that cultured Langerhans Cells do not proliferate *in vitro*. As stated above, applicants invention is a methoding of obtaining mature dendritic cells from proliferating dendritic cell precursors in the presence of factors that inhibit the proliferation or maturation of non-dendritic cell precursors. Therefore Hueffler does not render the claimed invention obvious.

Sallusto et al. was published in the Journal of Experimental Medicine in April 1994 (see bottom of page 1109). Attached as Exhibit 1, is a copy of the reference by Romani, et al. from the Journal of Experimental Medicine (1994) Volume 180:83-93. The subject matter of Romani et al. corresponds to the instantly claimed invention. For example, Table 2 of the present application (page 94) corresponds to Table 1 of Romani et al. and Figures 20 and 23 of the present application correspond to Figures 2 and 5 of Romani et al. Two of the authors listed on the Romani et al. reference, Ralph M. Steinman and Gerald Schuler, are inventors of the instant disclosure. On the bottom of page 91 of the Romani et al. reference, is a notation that the manuscript was received for publication February 17, 1994. Therefore, the instantly claimed invention was complete, prior to the publication of Sallusto et al. and Sallusto should be removed as a reference. Applicants will provide a §1.131 Declaration to properly remove this reference. Hence, Hueffler and Sallusto do not render the claimed invention obvious either alone or in combination. Reconsideration and withdrawal of this rejection is respectfully requested.

Rejection of Claims 10-12 Under 35 U.S.C. §103

Claims 10-12 are rejected under 35 U.S.C. §103 as being unpatentable over Hueffler et al. taken with Sallusto et al. as applied to claims 1-6, 8-9, and 20-22 above and further in view of Jakoby et al. Specifically, the Examiner contends:

"The combination of Hueffler et al. taken with Sallusto, et al. relied upon for the reasons discussed supra, teach utilization of GM-CSF and IL-4. These references differ from the claimed invention but not specifically indicating the exact concentration level of GM-CSF which is specifically claimed. However, it is well known in the art to adjust the concentration level of culture medium additives in order to optimize the experimental conditions for the particular

cell type being cultured. Jakoby, et al. on pages 75-77 teach that it is well known in the art of a cell culture to tailor media in order to optimize the experimental conditions. Each culture system requires examination of the particularly conditions that are best for the type of cell being studied by the investigator. Furthermore, each component of the system identified as a result effective variable to have its well recognized advantages for the purpose of optimizing the experimental conditions. This type of optimizing experimental conditions in well with the purview of the skilled artisan and is deemed a matter of routine experimentation. Accordingly, the claimed invention would have *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made, especially in the absence of sufficient clear and convincing evidence to the contrary."

Applicants traverse this rejection for the reasons discussed below.

As stated above, Jakoby et al. relates to general methods of culturing cells.

Jakoby et al. discusses types of culture systems, requirements for growth and some generalized standards for tissue culture. However, Jakoby et al. does not specifically discuss dendritic cell cultures or the requirements necessary to culture dendritic cells, such teachings are found only in the applicants' disclosure. Therefore, Jakoby et al. does not provide disclosure which remedies the deficiency of Hueffler et al. as discussed above. Sallusto should be removed as a reference as discussed above. Hence, the invention is not rendered obvious by this reference either alone or in combination with Hueffler et al. or Sallusto et al.

Rejection of Claims 7 and 13 Under 35 U.S.C. §103

Claims 7 and 13 stand rejected under 35 U.S.C. §103 as being unpatentable over Hueffler et al. taken with Sallusto et al. as applied to claims 1-6, 8-9 and 13 and 22 above and further in view of Koch et al. Specifically, the Examiner contends that:

"Koch et al. teaches that new insight into the biology of dendritic cells (DC) came from studies of murine epidermal Langerhans cells (LC) *in vitro*. Koch

et al. indicates that such studies can suggest that LC in the skin and DC in other non-lymphoid tissues represent precursors or immature elements of the dendritic cell system. Koch et al. teaches that the addition of TNF- α to murine epidermal Langerhans cells in culture allow such cells to maintain their viability. Therefore, in view of the teachings of Koch et al., one of ordinary skill in the art would have a reasonable expectation of success in maintaining viability of dendritic cells when TNF- α is added to the dendritic cell culture. Accordingly, one of ordinary skill in the art would have a reasonable expectation of success in adding TNF- α to the dendritic cell culture of the primary reference."

Applicants traverse this rejection for the reasons discussed below.

Koch et al. relates to the use of TNF- α in cultures of murine epidermal Langerhans Cells. Koch et al. does not relate to the production of mature dendritic cells from proliferating cultures of dendritic cells. Furthermore, Koch et al. neither teaches nor suggests the culturing of mature dendritic cells from dendritic cell precursors or the use of such factors in the cell culture which inhibit maturation or proliferation of non-dendritic cell precursors. Therefore, Koch et al. does not remedy the deficiency of Hueffler et al. as discussed above. As Sallusto should be removed as a reference, Koch et al. in combination with the other cited references does not render the claimed invention obvious and reconsideration is therefore respectfully requested.

Rejection of Claim 23 Under 35 U.S.C. §103

Claim 23 is rejected under 35 U.S.C. §103 as being unpatentable over Hueffler et al. taken with Sallusto et al. as applied to claims 1-6, 8-9 and 13-22 above and further in view of Ruley et al. Specifically, the Examiner contends:

"The combination of Hueffler et al. taken with Sallusto et al. differs from claim 23 by adding 10% fetal calf serum as opposed to 5% cord blood serum. However, Ruley et al. (U.S. Patent No. 5,364,783), column 22, lines 21-27

teaches us the use of cord blood serum in animal cell cultures is well known in the art. Therefore, it is deemed merely a matter of judicious selection on the part of the skilled artisan to utilize fetal calf serum or cord blood serum. Additionally, it is well known in the art to utilize anywhere from 1-20% of serum in animal cell cultures. Utilization of a particular concentration within that range is deemed merely a matter of routine optimization which is well within the purview of the skilled artisan."

Applicants traverse this rejection for the reasons discussed below.

As discussed above Hueffler et al. does not render the claimed invention obvious, and Sallusto et al. should be removed as a reference. Ruley, et al. relates to retroviral promotor trap vectors and provides general methods of culturing cells. However, Ruley et al. does not suggest methods of culturing dendritic cells or proliferating dendritic cells *in vitro* nor suggest factors that should be added to inhibit the maturation or proliferation of non-dendritic cell precursors. Absent such disclosures, Ruley et al. cannot render the claimed invention obvious. Hence, Ruley et al. either alone or in combination with the other cited references does not render the claimed invention obvious.

AUTHORIZATION

No additional fee is believed due.

The Commissioner is hereby authorized to charge any additional fees which may be required for this Amendment, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2016-4000US3. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

In the event that an extension of time is required or which may be required in addition to that requested in the petition and for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2016-4000US3.

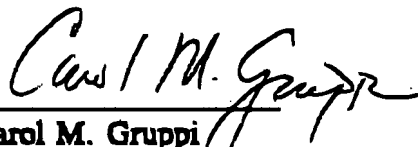
If any questions or issues remain or if the examiner has any comments or suggestions for expediting allowance of this application, he is urged to contact the undersigned at the telephone number below.

Respectfully submitted,

MORGAN & FINNEGAN

Dated: April 19, 1996

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